Original Article

Leptin-Receptor Polymorphisms Relate to Obesity through Blunted Leptin-Mediated Sympathetic Nerve Activation in a Caucasian Male Population

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Leptin plays a key role in the regulation of body weight through the sympathetic nervous system; however, the contributions of leptin-receptor polymorphisms to obesity and sympathetic nerve activity have not been fully clarified. In the present study, we examined the relationships between leptin-receptor polymorphisms, plasma leptin and whole-body norepinephrine (NE) spillover as an index of sympathetic nerve activity in a Caucasian male cohort. In 129 young healthy normotensive men with a wide range of body mass index (BMI) (19.4–39.5 kg/m²), we measured leptin-receptor polymorphisms (Gln223Arg, Lys656Asn, and Lys109Arg), plasma leptin levels, whole-body NE spillover, whole-body NE clearance, BMI and blood pressure (BP) levels in the supine position after overnight fasting. Overweight-obese (BMI ≥ 25 kg/m²) subjects had significantly greater BMI, BP levels, plasma leptin and whole-body NE spillover compared to lean (BMI < 25 kg/m²) subjects, but the NE clearance was similar. Overweight-obese subjects had significantly higher frequencies of the Arg223 allele and the Arg223 homozygous allele of Gln223Arg and the Asn656 allele of Lys656Asn compared to lean subjects. Subjects carrying the Arg223 homozygous or the Asn656 allele had higher levels of plasma leptin, BMI, waist circumference, and waist-to-hip ratio, but significantly less whole-body NE spillover, especially when they were also overweight-obese. BP levels and whole-body NE clearance were similar between subjects with and without the Arg223 homozygous or Asn656 allele. No differences were found in the distributions of the Arg109 allele of Lys109Arg polymorphism between nonobese and overweight-obese subjects. In addition, BMI, BP, plasma leptin levels, whole-body NE spillover and whole-body NE clearance were similar between those with and without the Arg109 allele. Together, these findings demonstrate that leptin-receptor polymorphisms were related to the incidence of obesity in a Caucasian male population. These polymorphisms were accompanied by high plasma leptin levels (leptin resistance) and lower whole-body plasma NE spillover (blunted sympathetic nerve activity). We therefore hypothesize that leptin-receptor play a role in the development of obesity through leptin resistance and blunted leptin-mediated sympathetic nerve activity. (*Hypertens Res* 2008; 31: 1093–1100)

Key Words: leptin-receptor polymorphisms, plasma leptin levels, sympathetic nerve activity, obesity, hypertension

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Introduction

Obesity is a growing health problem associated with a high risk of cardiac events. Obese humans have increased circulating plasma levels of the adipocyte hormone leptin (1), which is associated with heightened sympathetic nerve activity (2). Leptin increases thermogenesis and energy expenditure through stimulation of the sympathetic nervous system (3, 4). Leptin also increases norepinephrine (NE) turnover in brown adipose tissue (5) and white adipose tissue (6), mainly by enhancing the sympathetic nerve activity in these tissues. Thus, leptin plays a key role in the regulation of body weight through the sympathetic nervous system. The diminished effect of leptin on satiety is manifested as leptin resistance (7, 8). Leptin resistance might maintain obesity despite elevated leptin concentrations (5, 7–9).

Catecholamines are the major lipolytic hormones in human fat cells, and lipolytic catecholamine resistance has been observed in obesity. Studies on twins and in rare genetic disorders suggest a strong heredity component of catecholamine-induced lipolysis and resultant obesity (10). We have reported that obese men, especially those carrying the Gly16 allele of β 2-adrenoceptor polymorphisms accompanied with heightened sympathetic nerve activity, show blunted leptin stimulation on sympathetic nerve activity (plasma NE) compared to lean subjects (9). Thus, one could speculate that blunted sympathetic nerve activity–induced lipolysis or blunted leptin-mediated sympathetic nerve activation determined by genes might contribute to the obesity associated with high plasma leptin levels (leptin resistance).

Several clinical and epidemiological studies have demonstrated leptin receptor polymorphisms associated with obesity (3, 11–14). However, there have been few investigations on the concurrent relationships between leptin-receptor polymorphisms, plasma leptin levels and sympathetic nerve activity (15). In the present study, three leptin-receptor polymorphisms (Lys109Arg in exon 4, Gln223Arg in exon 6, and Lys656Asn in exon 14) from buffy coats (white blood cells) that may contribute to obesity (11–14) were studied in a Caucasian male population with a wide range of body mass indices (BMI). Further, we examined the relationships between the polymorphisms, plasma leptin levels, and whole-body plasma NE spillover as an index of sympathetic nerve activity. To our knowledge, this is the first study to examine the relationships between leptin-receptor polymorphisms, obesity (BMI), plasma leptin levels and sympathetic nerve activity concurrently to evaluate the relationships between leptin-receptor polymorphisms, the leptin–sympathetic nerve activity axis, and obesity.

Table 1. Characteristics in Lean Subjects (BMI < 25.0 kg/m²) vs. Overweight or Obese Subjects (BMI ≥ 25.0 kg/m²)

Variables	Lean subjects	Overweight + obese subjects
Subjects, <i>n</i>	40	89
Age, years	44.9 ± 10.2	47.8 ± 10.8
BMI, kg/m ²	22.0 ± 1.9	30.3 ± 3.5 [†]
Systolic BP, mmHg	123 ± 14	128 ± 14
Diastolic BP, mmHg	74 ± 11	76 ± 9
Mean BP, mmHg	90 ± 14	94 ± 11*
Heart rates, bpm	61 ± 13	62 ± 11
Waist, cm	91 ± 6	113 ± 8 [†]
Waist-to-hip ratio	0.81 ± 0.04	0.91 ± 0.06 [†]
Whole-body NE spillover, ng/min	431.6 ± 125.7	561.2 ± 186.8*
NE clearance, L/min	1.87 ± 0.38	2.01 ± 0.76
Leptin, ng/mL	4.48 ± 3.01	10.45 ± 5.18 [†]

BMI, body mass index; BP, blood pressure; NE, norepinephrine.

* $p < 0.05$, [†] $p < 0.01$ vs. lean subjects.

Methods

Subjects

One-hundred and twenty-nine non-smoking Caucasian men aged 23–59 years, with a BMI ranging from 19.4–39.5 kg/m² were recruited through newspaper advertisements. They consisted of 40 lean subjects (BMI < 25 kg/m²), 45 overweight subjects (25 ≤ BMI < 30 kg/m²) and 44 obese subjects (BMI ≥ 30 kg/m²) according to the overweight and obesity definitions by the Centers for Disease Control and Prevention (Atlanta, USA) (16). Subjects were excluded who had diabetes mellitus (fasting glucose level > 6.0 mmol/L), hypertension (≥ 140/90 mmHg), secondary hypertension, obstructive sleep apnea, cardiovascular or cerebrovascular disease, renal dysfunction or liver dysfunction. None of the subjects were taking medication for diabetes, hypertension, hyperlipidemia or other illnesses. The goal of the present study was to clarify the relationships among the genetic variance of the leptin receptor polymorphisms, plasma leptin levels, sympathetic nerve activity (whole-body NE spillover) and obesity (BMI). Thus, to minimize the influence of weight changes on sympathetic nerve activity and leptin levels (9, 17–19), all subjects had been weight stable for the preceding 6 months. The study was approved by the Alfred Hospital Human Research Ethics Committee. All participants provided written informed consent.

Measurements

Investigations were performed in a quiet, temperature-controlled room at the same time of the day (from 9:00 AM). After an overnight fast of > 12 h, BMI, blood pressure (BP),

Table 2. Comparisons of the Frequencies of Genotype of the Leptin-Receptor Polymorphisms between Lean Subjects (BMI<25.0 kg/m²) and Overweight or Obese Subjects (BMI≥25.0 kg/m²)

	Genotype	Lean subjects (n (%))	Overweight or obese subjects (n (%))	χ^2 test	
				For genotype	For allele
Lys109Arg	Arg/Arg	2 (5.0)	1 (1.1)	$\chi^2=5.31, p=0.379$	$\chi^2=2.92, p=0.405$
	Arg/Lys	12 (30.0)	26 (29.2)		
	Lys/Lys	26 (65.0)	62 (69.7)		
Gln223Arg	Gln/Gln	18 (45.0)	25 (28.1)	$\chi^2=10.52, p=0.062$ (vs. Arg223 homozygous, $\chi^2=11.84, p=0.008$)	$\chi^2=10.96, p=0.012$
	Gln/Arg	17 (42.5)	38 (42.7)		
	Arg/Arg	5 (12.5)	26 (29.2)		
Lys656Asn	Lys/Lys	21 (52.5)	32 (36.0)	$\chi^2=8.11, p=0.1505$	$\chi^2=8.303, p=0.040$
	Lys/Asn	15 (37.5)	39 (43.8)		
	Asn/Asn	4 (10.0)	18 (20.2)		

BMI, body mass index.

heart rate, and venous sampling for the extraction of genomic DNA from leukocytes were taken after 30 min rest in the supine position. Subjects abstained from caffeine for 12 h, alcohol for 24 h, and exercise for 36 h. BP and heart rate were measured ≥ 3 times in the supine position using an automated sphygmomanometer (DINAMAP 1846-SX Blood Pressure Monitor; Critikon Corp., Tampa, USA) with a cuff size that was adjusted for arm circumference, and were averaged. The accuracy of the automated sphygmomanometer was tested against a mercury sphygmomanometer before use.

Laboratory Determinations

Whole-body sympathetic nerve activity was assessed by measurement of the apparent rate of appearance of endogenous NE in plasma (NE spillover rate) using the isotope dilution technique (20, 21). The plasma concentrations of neurochemicals were determined by HPLC with electrochemical detection. The intra-assay coefficients of variations in our laboratory were 3% for NE and 7% for [³H]NE; the inter-assay coefficient of variations were 11% and 6%, respectively. Plasma leptin levels were determined by RIA (Linco Research, Inc., St. Charles, USA).

Genotyping

Three single-nucleotide polymorphisms (SNPs) (rs1137100, rs1137101, rs8179183) were genotyped by pre-developed TaqMan allelic discrimination assays (Applied Biosystems, Foster City, USA). The PCR was carried out in a total reaction volume of 5 μ L using the following amplification protocol: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 60 s. Allelic discrimination was measured automatically on an ABI Prism HT7900 (Applied Biosystems) using the Sequence Detection System Version 2.1 software.

Statistical Analysis

The genotype distribution of the polymorphisms was tested for Hardy-Weinberg equilibrium by χ^2 analysis. Values are shown as the mean $\pm$ SD. Differences among groups were examined by an unpaired *t*-test. All data analyses were performed with the program SPSS 8.0 for Windows (SPSS Inc., Chicago, USA). Values of $p < 0.05$ were considered significant.

Results

Characteristics of Lean Subjects (BMI<25.0 kg/m²) vs. Overweight or Obese Subjects (BMI≥25.0 kg/m²)

The overweight or obese group had significantly greater BMI, BP levels, waist circumference, waist-to-hip ratio, whole-body NE spillover and plasma leptin compared to the lean group. The NE clearance rate, however, was similar between the two groups (Table 1).

Comparisons of the Frequencies of the Leptin-Receptor Polymorphisms (Lys109Arg, Gln223Arg, and Lys656Asn) between Lean Subjects (BMI<25.0 kg/m²) vs. Overweight or Obese Subjects (BMI≥25.0 kg/m²)

Overweight or obese subjects had significantly higher frequencies of the Arg223 homozygous allele of the Gln223Arg polymorphism and the Asn656 allele of the Lys656Asn polymorphism. However, the allele distributions of Lys109Arg were similar between lean and overweight or obese subjects (Table 2).

The Hardy-Weinberg equilibriums were $p=0.6398$ for Lys109Arg, $p=0.1122$ for Gln223Arg, and $p=0.2055$ for Lys656Asn.

Table 3. Characteristics of the Subjects Carrying the Arg223 Homozygous and Carrying the Gln223 Allele of the Gln223Arg Polymorphism

Variables	Gln223/Gln223	Gln223/Arg223	Arg223/Arg223	With Gln223 allele	
				(without Arg223 homozygous)	With Arg223 allele
Subjects, <i>n</i>	43	55	31	98	86
Age, years	46.5±8.2	48.3±11.1	44.7±10.2	47.5±9.8	47.0±10.4
BMI, kg/m ²	24.8±5.7*	27.6±4.8	31.9±8.9‡	26.4±7.4*	29.2±6.9‡
Systolic BP, mmHg	128±14	125±13	126±15	126±14	126±14
Diastolic BP, mmHg	76±11	75±9	76±8	75±10	76±9
Mean BP, mmHg	90±11	91±12	94±10	91±13	92±12
Heart rates, bpm	63±15	61±11	60±10	62±13	61±11
Waist, cm	93±13‡	110±9‡	119±10§	103±10*	113±10§
Waist-to-hip ratio	0.85±0.06*	0.87±0.05	0.92±0.06‡	0.86±0.07*	0.89±0.07
Whole-body NE spillover, ng/min	568.7±123.5*	523.1±131.2	451.3±105.3‡	543.1±116.1*	497.3±124.3
NE clearance, L/min	2.26±0.90	1.90±0.55	1.69±0.58	2.06±0.72	1.82±0.51
Leptin, ng/mL	5.32±2.63‡	7.74±3.73	14.68±3.70§	6.68±4.79‡	10.21±4.81‡

BMI, body mass index; BP, blood pressure; NE, norepinephrine. * $p<0.05$, † $p<0.01$ vs. Arg223/Arg223; ‡ $p<0.05$, § $p<0.01$ vs. Gln223/Gln223.

Table 4. Characteristics of the Subjects with and without the Gln223 Allele of Gln223Arg Polymorphism in Lean Subjects (BMI<25.0 kg/m²) vs. Overweight or Obese Subjects (BMI≥25.0 kg/m²)

Variables	Lean subjects		Overweight+obese subjects	
	With Gln223 allele	With Arg223 homozygous	With Gln223 allele	With Arg223 homozygous
Subjects, <i>n</i>	35	5	63	26
Age, years	44.4±8.7	48.4±7.0	49.2±8.9	44.0±10.1
BMI, kg/m ²	21.9±2.0	22.7±0.9	28.9±2.5‡	33.7±3.3‡,§
Systolic BP, mmHg	123±13	123±14	129±12*	127±11
Diastolic BP, mmHg	74±9	74±7	76±9	77±10
Mean BP, mmHg	90±12	90±11	94±10*	94±9
Heart rates, bpm	61±11	61±9	62±11	60±10
Waist, cm	90±8	92±9	110±9‡	123±7‡,§
Waist-to-hip ratio	0.80±0.05	0.87±0.05‡	0.88±0.09‡	0.95±0.08‡,§
Whole-body NE spillover, ng/min	457.6±102.3	249.6±101.6‡	590.6±152.0*	490.1±100.7*‡,§
NE clearance, L/min	1.79±0.33	2.43±0.60	2.21±0.91	1.55±0.81
Leptin, ng/mL	4.02±3.25	7.70±3.45	8.15±3.77‡	16.01±6.67‡,§

BMI, body mass index; BP, blood pressure; NE, norepinephrine. * $p<0.05$, † $p<0.01$ vs. lean subjects; ‡ $p<0.05$, § $p<0.01$ vs. subjects with Gln223 allele.

The findings that overweight or obese subjects had significantly higher frequencies of the Arg223 allele (Arg223 homozygous) of Gln223Arg and the Asn656 allele of Lys656Asn, but not the Arg109 allele of Lys109Arg, indicates that the Arg223 allele of Gln223Arg and Asn656 allele of Lys656Asn (but not Lys109Arg) are closely linked to overweight-obesity. Thus, we compared the parameters between subjects with and without the Arg223 allele (especially Arg223 homozygous) of Gln223Arg or the Asn656 allele of Lys656Asn.

Characteristics of the Subjects Carrying the Arg223 Allele of Gln223Arg Polymorphism

Subjects carrying the Arg223 allele had significantly greater BMI, waist circumference and plasma leptin levels, and slightly lower whole-body NE spillover compared to those carrying the Gln223 homozygous allele (those without the Arg223 allele) (Table 3). Subjects carrying the Arg223 homozygous allele of Gln223Arg had significantly greater BMI, waist circumference, waist-to-hip ratio, and plasma leptin levels compared to subjects carrying the Gln223 allele

Table 5. Characteristics of the Subjects Carrying the Asn656 Allele of the Lys656Asn Polymorphism

Variables	Lys656/Lys656	Lys656/Asn656	Asn656/Asn656	With Asn656 allele
Subjects, <i>n</i>	53	54	22	76
Age, years	47.2±10.1	46.7±9.8	45.3±9.1	46.1±9.6
BMI, kg/m ²	25.8±4.9 [‡]	28.4±2.4	30.6±3.7*	29.5±4.9*
Systolic BP, mmHg	127±11	127±12	123±15	125±14
Diastolic BP, mmHg	75±11	75±5	80±8	77±7
Mean BP, mmHg	90±14	92±7	94±10	90±13
Heart rates, bpm	61±10	63±14	60±10	62±15
Waist, cm	95±9 [‡]	112±9*	121±14*	115±12 [‡]
Waist-to-hip ratio	0.83±0.11 [‡]	0.88±0.09	0.97±0.10*	0.91±0.08*
Whole-body NE spillover, ng/min	561.1±194.4 [‡]	503.4±111.3 [‡]	457.8±103.0*	487.9±111.7*
NE clearance, L/min	2.00±0.72	1.93±0.54	1.99±0.27	1.95±0.39
Leptin, ng/mL	6.97±4.33 [‡]	9.28±3.02	10.09±4.61*	9.74±2.61*

BMI, body mass index; BP, blood pressure; NE, norepinephrine. * $p < 0.05$, [‡] $p < 0.01$ vs. Lys656/Lys656; [‡] $p < 0.05$ vs. Asn656/Asn656.

Table 6. Characteristics of the Subjects with and without the Asn656 Allele of Lys656Asn Polymorphism in Lean Subjects (BMI < 25.0 kg/m²) vs. Overweight or Obese Subjects (BMI ≥ 25.0 kg/m²)

Variables	Lean subjects		Overweight+obese subjects	
	Without Asn656 allele	With Asn656 allele	Without Asn656 allele	With Asn656 allele
Subjects, <i>n</i>	21	19	32	57
Age, years	46.1±9.0	48.5±5.0	47.9±9.0	47.6±8.7
BMI, kg/m ²	21.7±2.1	22.3±0.8	28.5±6.1 [†]	31.3±3.5 ^{†,‡}
Systolic BP, mmHg	123±15	123±7	130±9*	127±10*
Diastolic BP, mmHg	73±9	75±7	76±9	77±10
Mean BP, mmHg	89±13	91±9	94±9*	96±10
Heart rates, bpm	61±10	61±9	61±12	62±10
Waist, cm	89±10	99±5 [‡]	101±9*	122±7 ^{†,§}
Waist-to-hip ratio	0.81±0.06	0.83±0.04	0.88±0.09*	0.94±0.07 [†]
Whole-body NE spillover, ng/min	445.0±115.6	416.8±125.7	628.5±159.9 [†]	518.5±137.5* [‡]
NE clearance, L/min	1.83±0.31	1.91±0.57	2.11±0.88	1.96±0.91
Leptin, ng/mL	4.10±2.78	4.92±2.22	8.85±3.95 [†]	11.35±6.01 ^{†,‡}

BMI, body mass index; BP, blood pressure; NE, norepinephrine. * $p < 0.05$, [†] $p < 0.01$ vs. lean subjects; [‡] $p < 0.05$, [§] $p < 0.01$ vs. subjects without Asn656 allele.

(those without the Arg223 homozygous allele), but whole-body NE spillover was significantly less in subjects carrying the Arg223 homozygous allele. Subjects carrying the Arg223 homozygous allele had similar whole-body NE clearance rates, BP levels and heart rates to those carrying the Gln223 allele (Table 3).

Subjects carrying the Arg223 homozygous allele of Gln223Arg, especially within the overweight or obese group, had significantly greater BMI, waist circumference, waist-to-hip ratio, and plasma leptin levels compared to subjects carrying the Gln223 allele. Whole-body NE spillover was significantly lower in subjects carrying the Arg223 homozygous allele, either within the lean or the overweight or obese group, compared to those with the Gln223 allele (Table 4).

Characteristics of the Subjects Carrying the Asn656 Allele of the Lys656Asn Polymorphism

BMI, waist circumference and plasma leptin levels were significantly greater in subjects carrying the Asn656 allele of Lys656Asn, but whole-body NE spillover rates were significantly lower. NE clearance rates, BP levels and heart rates were similar between subjects with and without the Asn656 allele (Table 5).

In both the lean and overweight or obese subjects carrying the Asn656 allele, BMI, waist circumference and plasma leptin levels were greater than in those without the Asn656 allele, but whole-body NE spillover was significantly lower in overweight or obese subjects carrying the Asn656 allele (Table 6).

Discussion

The main findings in the present study were that overweight or obese subjects had significantly higher frequencies of the Arg223 homozygous allele of the Gln223Arg leptin-receptor polymorphism and the Asn656 allele of the Lys656Asn leptin-receptor polymorphism, although the number of subjects studied was relatively small ($n=129$). Further, those subjects carrying the leptin-receptor polymorphisms had significantly higher levels of plasma leptin, BMI, and waist circumference (central obesity), but significantly lower sympathetic nerve activity shown as significantly lower whole-body NE spillover. It should be noted that this is the first study to examine the relationships between leptin-receptor polymorphisms, obesity (BMI), plasma leptin levels and sympathetic nerve activity concurrently to evaluate the relationships between leptin-receptor polymorphisms and the leptin-sympathetic nerve activity axis in obesity.

Leptin was initially touted as a major suppressor of appetite based on the finding that total deficiency of leptin or its receptor leads to hyperphagia and obesity in mice and humans (22, 23). Leptin links adipose tissue mass to food intake and energy expenditure through a negative feedback loop by binding and activating leptin-specific receptors in the hypothalamus (24–26). Further, leptin receptors are expressed in the nervous system and peripheral tissue, including adipose tissue, skeletal muscle, pancreatic β -cells, and the liver (22, 23, 25). Exogenous administration of leptin has been reported to decrease body weight, body fat, and food intake, and to increase energy expenditure accompanied with sympathetic nervous activation in animals and humans (27–31). However, as body fat determines the circulating leptin concentrations, obese individuals generally have increased levels of leptin (32), prompting the hypothesis that “leptin resistance” plays an important role in the development of obesity in humans (7, 8). Importantly, it has been suggested that leptin responsiveness to energy restriction is affected by the functionality of the leptin receptor.

Several investigators have demonstrated that leptin-receptor polymorphisms predict obesity (3, 14, 33) and weight change (12, 34), and that leptin-receptor polymorphisms might play a role in the acute leptin reduction after energy restriction (35). The contributions of the leptin-receptor polymorphisms Lys109Arg, Gln223Arg, and Lys656Asn on obesity were analyzed (36). On the other hand, other investigators have observed no linkages between leptin-receptor polymorphisms and obesity in Pima Indians (37) and in French subjects (38). The contributions of leptin-receptor polymorphisms to obesity are thus controversial. A Caucasian population was reported to have lower frequencies of the Arg223 and Arg109 alleles and higher frequency of the Asn656 allele compared to an Asian population (36), demonstrating that the allele distributions of Lys109Arg, Gln223Arg, and Lys656Asn are different among ethnic

groups. Thus, it is possible that these conflicting results may be due in part to differences in the ethnicity (14), gender (14, 39), severity of obesity, and dietary status (35) of the studied populations. Further, a weak association might have been dismissed due to the relatively limited numbers of subjects studied (36). In the present study we evaluated the relationships between three leptin-receptor polymorphisms (Lys109Arg, Gln223Arg, and Lys656Asn) and obesity (BMI) in 129 middle-aged Caucasian men with a wide range of BMI. Interestingly, our findings are in good accordance with the HERITAGE Family Study, which has shown that the Arg223 allele of Gln223Arg is associated with heavier BMI, fat mass, percent fat mass, and leptin levels only in the middle-aged male Caucasian population, but not in blacks or female Caucasian populations (14).

Obesity is considered to result from reduced energy expenditure, which results in a reduction of resting metabolic rate, and a negative energy balance (thermogenesis) through diminished sympathetic nerve activity. Sympathetic nerve activity plays an important role in stimulating lipolysis by catecholamines in white adipose tissue in humans and in the development of obesity. However, many clinical and epidemiological studies have shown heightened sympathetic nerve activity in obesity as well as higher levels of plasma leptin (2, 9, 17–19). In the present study, sympathetic nerve activity in subjects without the Arg223 homozygous allele and Asn656 allele was significantly higher than in those carrying the Arg223 homozygous allele or Asn656 allele, although those without the Arg223 homozygous allele or Asn656 allele had lower levels of plasma leptin, BMI and waist circumference. These results demonstrate that subjects carrying the Arg223 homozygous or Asn656 allele, which are closely linked to obesity, have relatively blunted leptin-mediated sympathetic nerve activity (observed as lower whole-body NE spillover) leading to lower thermogenesis, which may result in obesity (central obesity). Blunted leptin-mediated sympathetic nerve activity might play an important role in obesity, as we have previously reported (9, 40). However, we could not discuss the relationship of leptin-receptor polymorphisms in thermogenesis or energy expenditure because we did not measure resting metabolic rates or energy expenditure in the present study. One could speculate that subjects carrying leptin-receptor polymorphisms might be prone to obesity because of reductions in energy expenditure preceded by low sympathetic nerve activity (12).

Leptin resistance, which is defined as an impairment in the action of leptin accompanied by hyperleptinemia, is a common feature of obesity in animal models and in humans (7, 41, 42). Therefore, hyperleptinemia has been used as a surrogate index for leptin resistance to the adipose tissue mass (42). Experimental results show that chronic systemic and intracerebral administration of leptin increases BP levels or heart rates in animal models (4, 41). Leptin produces sympathetic nerve activation in the kidneys, adrenal glands and brown adipose tissue, indicating that the obesity-associated

increases in sympathetic nerve activity could be due in part to these various sympathetic effects of leptin (4, 5, 8). In other words, leptin resistance selectively affects the metabolic actions of leptin, but spares leptin-mediated sympathetic activation. In the present study, the findings that obese subjects, irrespective of whether or not they had the leptin-receptor polymorphisms, had higher whole-body NE spillover and BP levels might be linked to "selective leptin resistance" (7, 8). On the other hand, the subjects carrying the Arg223 homozygous or Asn656 allele had higher leptin levels, BMI and waist circumference compared to those without these polymorphisms, but BP levels and heart rates were similar. The absence of any linkage between leptin-receptor polymorphisms, plasma leptin levels, and BP levels might have been caused by the relatively lower sympathetic nerve activity associated with these polymorphisms, because higher BP levels associated with high sympathetic nerve activity were observed in overweight or obese subjects compared to lean subjects, even in those carrying these polymorphisms. These findings might help to explain why not all obese subjects have hypertension.

The Hardy-Weinberg equilibrium demonstrated the subjects in this study had a normal distribution ($p > 0.05$) of the three polymorphisms studied, but we cannot rule out the possibility of a chance variation due to the small number of subjects ($n = 129$). The findings that Lys656Asn polymorphism was not associated with overweight-obesity or blunted sympathetic nerve activity may have been related to the small size of the cohort. The distribution of the Arg109 homozygous allele was very low ($n = 3$) in this cohort.

In summary, a strong association of leptin-receptor polymorphisms with obesity, but not BP levels, was observed in a middle-aged Caucasian male population. Blunted leptin-mediated sympathetic nerve activity (observed as whole-body NE spillover) related with leptin-receptor polymorphisms may play a major role in the maintenance of obesity through lower thermogenesis. Further, the absence of a link between leptin-receptor polymorphisms and BP levels may have been related with the relatively lower sympathetic nerve activity. Further study will be needed to clarify the differences in the contributions of leptin-receptor polymorphisms to BMI (obesity) and BP levels (hypertension).

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